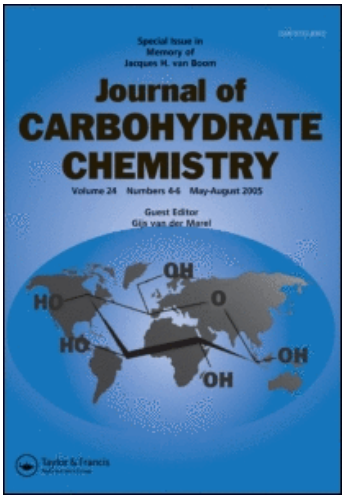

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Recognition Properties of Processing α-Glucosidase I and α-Glucosidase II

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ABSTRACT

All four possible monodeoxy derivatives of *p*-nitrophenyl α-D-glucopyranoside (PNP Glc) and 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol derivatives were prepared and used as substrates and inhibitors of rat liver processing α-glucosidases. α-Glucosidase II hydrolyzed the 2-deoxy derivative of PNP Glc (**1**); the hydrolysis of **1** was more rapid than that of PNP Glc. These results indicate that the presence of a C-2 hydroxyl group is not essential for the action of α-glucosidase II. In contrast, PNP Glc and all of the deoxy derivatives of PNP Glc **1**–**4** inhibited α-glucosidase I. These results indicate that α-glucosidase I does not necessarily need all of the hydroxyl groups of the glycon moiety for binding to the enzyme. 2,6-Anhydro-1-benzamide-D-*glycero*-D-*ido*-heptitol (**11**), with a terminal phenyl group, inhibited α-glucosidase I and α-glucosidase II. Both α-glucosidase I and II showed the same aglycon specificities. When probes **5**–**12** were assayed for their ability to inhibit processing by α-glucosidases at the cellular level, no effects on glycoprotein processing were observed.

Key Words: Processing glucosidase; Inhibitor; Substrate specificity; Deoxy glucoside.

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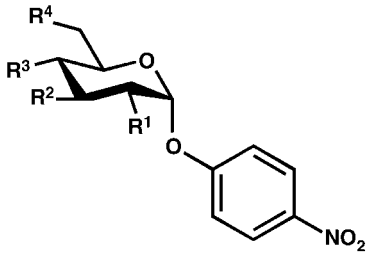
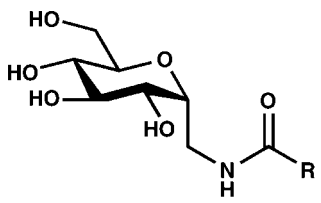
INTRODUCTION

Endoplasmic reticulum processing α-glucosidases are key enzymes in the biosynthesis of asparagine-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins. These enzymes are a target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion, and infectivity.[1–3] Substrate specificities are used to categorize enzymes and are important in designing structure-based inhibitors. We previously elucidated the glycon specificities of α-glucosidases (EC 3.2.1.20),[4–6] α-galactosidases (EC 3.2.1.22),[5,7] and α-mannosidases (EC 3.2.1.24)[5,8] against all possible monodeoxy and monomethyl derivatives of the corresponding *p*-nitrophenyl α-D-glycopyranosides, and confirmed that some of them were good substrates for the enzymes. Next, we planned to investigate the glycon and aglycon specificities of the processing α-glucosidases I (EC 3.2.1.106), and α-glucosidases II (EC 3.2.1.84), in rat liver microsomes using synthetic probes **1**–**12** (Fig. 1). Four probes **1**–**4** were synthesized previously.[4,7,8] Thus, these studies required the efficient synthesis of probes **5**–**12**.

These latter eight probes included 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol, which might mimic to a great extent the topography of α-D-glucopyranoside and modified aglycon of α-glucopyranoside.

In this report, we first describe the synthesis of probes **5**–**12** with modified aglycons. These compounds were evaluated with regard to their ability to inhibit processing α-glucosidase I and α-glucosidase II. All of the monodeoxy analogs of *p*-nitrophenyl α-D-glucopyranoside (PNP Glc) **1**–**4**, available from previous studies, were evaluated as substrates and inhibitors of both enzymes. Finally, probes **5**–**12** were also tested in a cell culture system. We divided the recognition properties of α-glucosidase I and α-glucosidase II into glycon-specific and aglycon-specific and discuss our findings.





1: R^1=H, R^2=R^3=R^4=OH
2: R^1=R^3=R^4=OH, R^2=H
3: R^1=R^2=R^4=OH, R^3=H
4: R^1=R^2=R^3=OH, R^4=H

5: R=CH_2OH
6: R=$(CH_2)_2OH$
7: R=$(CH_2)_3OH$
8: R=$(CH_2)_4OH$

9: R=$(CH_2)_5OH$
10: R=Me
11: R=Ph
12: R=$C(CH_3)_3$

Figure 1. Chemical structure of probes **1**–**12**.

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RESULTS AND DISCUSSION

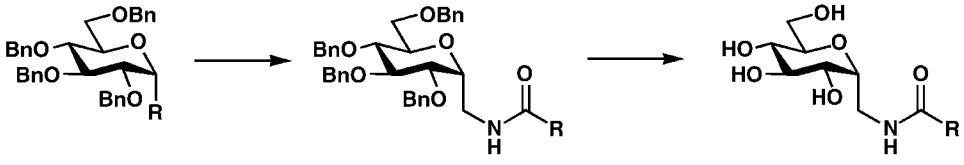
Synthesis of Heptitol Derivatives

The synthesis of the 1-amino-2,6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol derivatives (**5**–**12**) that were used in the present study is presented in Sch. 1. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl cyanide (**13**)[9] was used as a starting material for thesynthesis of **5**–**12**. Compound **14**[9] was coupled with acetoxyacetic acid, 3-hydroxypropionic acid, 4-hydroxybutyric acid, glutaric acid monomethyl ester, and adipic acid monomethyl ester to give **15**, **17**–**19**, and **21**, respectively.[10] Compound **15** was treated with a base to afford **16**. Compounds **19** and **21** were treated with lithium aluminum hydride (LAH) in tetrahydrofuran (THF) to give **20** and **22**, respectively. Compound **14** was acylated with acetic anhydride, benzoyl chloride, and pivaloyl chloride in pyridine to give **23**–**25** in good yield, respectively. Treatment of the resulting amides (**16**–**18**, **20**, and **22**–**25**) with palladium hydroxide on carbon gave probes **5**–**12** in good yields. To the best of our knowledge, there have been no previous reports on the synthesis of probes **5**–**12**.

Specificities of Processing α-Glucosidases

D. M. Burns et al. have been reported that rat liver α-glucosidase II showed hydrolytic activity against PNP Glc.[11] We investigated the specific activity of rat liver microsome α-glucosidase II in the hydrolysis[11–14] of PNP Glc and its deoxy derivatives **1**–**4**. Clearly, of the four deoxy derivatives of PNP Glc, α-glucosidase II hydrolyzed only the 2-deoxy glucopyranoside (**1**); its activity with **1** appeared to be substantially higher than that with PNP Glc. Kinetic studies of the hydrolysis of PNP Glc and **1** were also carried out (Table 1). The V_{max}/K_m value of α-glucosidase II for **1** was 3.7-fold greater than for PNP Glc, which indicated that probe **1** was a good substrate for the enzyme. These results also indicated that the C-2 hydroxyl group of the α-glucopyranoside residue is not indispensable for recognition between the substrate and α-glucosidase II.

In assays of the inhibitory effects of PNP Glc and probes **1**–**12** against processing α-glucosidase I and α-glucosidase II in rat liver microsomes, we used [^{3}H]glucose-labeled



13: R=CN
14: R=CH_2NH_2

15: R=CH_2OAc
16: R=CH_2OH
17: R=$(CH_2)_2OH$
18: R=$(CH_2)_3OH$
19: R=$(CH_2)_3COOMe$
20: R=$(CH_2)_4OH$

21: R=$(CH_2)_4COOMe$
22: R=$(CH_2)_5OH$
23: R=CH_3
24: R=Ph
25: R=$(CH_3)_3$

5: R=CH_2OH
6: R=$(CH_2)_2OH$
7: R=$(CH_2)_3OH$
8: R=$(CH_2)_4OH$
9: R=$(CH_2)_5OH$
10: R=CH_3
11: R=Ph
12: R=$C(CH_3)_3$

Scheme 1. Synthesis of probes **5**–**12**.

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Table 1. Hydrolytic activity of processing α-glucosidase II.

Substrate	Relative rate of hydrolysis (%)	k_m (mM)	V_{max} (μmol/min/unit)	V_{max}/k_m
PNP α-D-glucopyranoside	100	0.92	1.12	1.23
PNP 2-deoxy-α-D-glucopyranoside (**1**)	189	0.76	3.44	4.53
PNP 3-deoxy-α-D-glucopyranoside (**2**)	<1	—	—	—
PNP 4-deoxy-α-D-glucopyranoside (**3**)	<1	—	—	—
PNP 6-deoxy-α-D-glucopyranoside (**4**)	<1	—	—	—

Note: Relative rate of hydrolysis was expressed by comparison with the amount of *p*-nitrophenol that was released from PNP Glc by α-glucosidase II, which was taken as 100%.

vesicular stomatitis virus glycoprotein (VSV G)[11–16] and PNP Glc as substrates, and the results are summarized in Table 2. PNP Glc and probes **1**–**4** inhibited α-glucosidase I. At 5 mM, PNP Glc and probes **1**–**4** inhibited α-glucosidase I by 56.2%, 71.7%, 18.5%, 22.2%, and 32.3%, respectively. On the other hand, PNP Glc and probes **1**–**4** at 5 mM did not inhibit α-glucosidase II. It has been reported that both PNP Glc and β-D-glucopyranoside inhibit purified mammary-gland α-glucosidase I, with 19% and *p*-nitrophenyl 14% inhibition at 0.5 μM.[17] These findings indicate that the C-2, C-3, C-4, and C-6 hydroxyl groups of the α-D-glucopyranoside residue are important for recognition of the substrate by α-glucosidase I. However, the C-2 hydroxyl group of the α-D-glucopyranoside residue is not critical for substrate recognition. The failure of the 3-, 4- and 6-deoxy derivatives

Table 2. Inhibitory activity against processing α-glucosidases.

	Enzyme	
Inhibition (%)	Glucosidase I	Glucosidase II
PNP Glc	56.2	HD
1	71.7	HD
2	18.5	<1.0
3	22.2	<1.0
4	32.3	<1.0
5	<1.0	<1.0
6	<1.0	<1.0
7	<1.0	<1.0
8	<1.0	<1.0
9	<1.0	<1.0
10	<1.0	<1.0
11	18.2	5.9
12	<1.0	<1.0

Note: Probe concentrations: Probes **1**–**4**: 5 mM; probes **5**–**12**: 2 mM. Substrate of glucosidase I: [^{3}H]Glucose labeled VSV G. Substrate of glucosidase II: PNP Glc. HD: Hydrolyzing activity was observed.

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(**2**, **3**, and **4**) to inhibit α-glucosidase II suggests that the C-3, C-4, and C-6 hydroxyl groups of α-D-glucopyranoside are important for substrate recognition by α-glucosidase II. Compound **11**, with a terminal phenyl group, moderately inhibited both enzymes. At 2 mM compound **11** inhibited α-glucosidase I by 18% and α-glucosidase II by 6%. Both α-glucosidase I and II showed the same aglycon specificities. Probes **5**–**12** were assayed with regard to their ability to inhibit glycoprotein processing at the cellular level. The VSV G was prepared from VSV-infected and probe-treated baby hamster kidney (BHK) cells. Analyses of the *N*-glycan structure of obtained VSV G using endo H, which is known to have hydrolytic activity against high-mannose type *N*-glycan, failed to confirm that these compounds inhibited processing α-glucosidases (data not shown).

Asano et al. and Muroi et al. reported that fagomine (a 2-deoxy derivative of 1-deoxynojirimycin) and 1-azafagomine (in which the C-1 methylene group of fagomine is replaced by an –NH– group) inhibited processing α-glucosidases in rat liver microsomes.[14,16] Based on these inhibitory activities and our data, fagomine and 1-azafagomine are considered to inhibit α-glucosidase II rather than α-glucosidase I.

We previously reported that the glycosyl hydrolase family 31 α-glucosidases from rice, sugar beet, flint corn, and *Aspergillus niger* had 2-deoxyglucosidase activity; its activity against **1** appeared to be substantially higher than that against PNP Glc.[4,6] Therefore, rat liver microsome α-glucosidase II may have properties similar to those found in glycosyl hydrolase family 31.

EXPERIMENTAL

General Methods

Melting points were determined with a BÜCHI 510 capillary apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-370 digital porlarimeter at 25°C. The NMR spectra were recorded with a JEOL α-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts were expressed in ppm downfield shift from Me_4Si. Where appropriate, signal assignments were deduced by COSY and HMQC NMR experiments. Low- and high-resolution mass spectra were obtained with a JEOL JMS AX505WA instrument under positive ion FAB conditions. Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck, Tokyo, Japan). The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 F_{254} (0.25 mm, Merck, Tokyo, Japan).

Method A

To the solution of compound **13**[9] (1.6 mmol), in THF (30 mL), a suspension of LAH (3 mmol) in THF (5 mL) was slowly added. The mixture was stirred at room temperature for 1 hr and then quenched with ethyl acetate (AcOEt). The resulting mixture was diluted with satd Na_2SO_4aq and extracted with diethyl ether (Et_2O). Evaporation of the Et_2O phase gave 1-amino-2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-D-*glycero*-D-*ido*-heptitol (**14**) (1.51 g, 94.4%) as unstable syrup.

Method B

To the solution of compound **14** (0.63 mmol) in dichloromethane (CH_2Cl_2, 5 mL) and the mixture was added triethylamine (NEt_3, 1.9 mmol), carboxylic acid (0.94 mmol) in CH_2Cl_2 (1 mL), and diphenylphosphoryl azide (0.94 mmol) in CH_2Cl_2 (1 mL). After stirring the mixture at 0°C for 8 hr. The resulting mixture was diluted with 1 M HClaq and extracted with Et_2O (2 × 80 mL). The organic extract was washed with satd $NaHCO_3$aq, and brine, and then concentrated after drying over Na_2SO_4.

Method C

To the solution of compound **14** (1.38 mmol) in *N*,*N*-dimethylformamide (DMF, 15 mL) and the mixture was added NEt_3 (3.77 mmol), carboxylic acid (1.88 mmol) in DMF (2 mL) and diethylphosphoryl cyanide (1.88 mmol) in DMF (2 mL) was added.[10] After stirring the mixture at 0°C for 18 hr. The resulting mixture was diluted with 1 M HClaq and extracted with Et_2O (3 × 50 mL). The organic extract was washed with satd $NaHCO_3$aq, and brine, and then concentrated after drying over Na_2SO_4.

Method D

To the solution of methylester derivative (0.07 mmol), in THF (10 mL), a suspension of LAH (0.14 mmol) in THF (2 mL) was slowly added. The mixture was stirred at room temperature for 2 hr and then quenched with AcOEt. The resulting mixture was diluted with satd Na_2SO_4aq and extracted with Et_2O. The organic extract was washed with satd $NaHCO_3$aq, and brine, and then concentrated after drying over Na_2SO_4.

Method E

To the solution of compound **14** (1.0 mmol), in pyridine (20 mL) at room temperature was added acyl halide (4.0 mmol). The mixture was stirred for overnight and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd $NaHCO_3$aq, and brine, and then dried over Na_2SO_4. Removal of solvent afforded the corresponding amide.

Method F

A mixture of benzyloxy derivative (0.3 mmol) in ethanol (EtOH, 10 mL) and 1 M HClaq (50 μL) was hydrogenated under H_2 with 20% palladium hydroxide on carbon (20 mg). After stirring the mixture for 1 hr, the palladium charcoal was removed by filtration through Celite and the solvent was concentrated.

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Preparation of Probes

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxoethyl-2-acetoxy) amino]-D-*glycero*-D-*ido*-heptitol (**15**)

According to methods A and B, compound **15** was prepared from **13** (1.14 g, 2.08 mmol). The product was purified by column chromatography on silica gel (1 : 1 hexane–AcOEt) to afford 0.44 g (32.6%) of **15**, which was recrystallized from hexane–AcOEt: $[\alpha]_D$ +27.8° (*c* 3.6, $CHCl_3$); mp 86–87°C; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H, $-OCOCH_3$), 3.42–3.81 (m, 8H, H-1, H-3, H-4, H-5, H-6, and H-7), 4.09 (m, 1H, H-2), 4.46–4.92 (4 AB system, 8H, $-CH_2$-Ph), 4.51 (s, 2H, $-COCH_2O-$), 6.37 (t, 1H, NH), 7.13–7.36 (m, 20H, aromatic H); MS: 654 (MH^+).

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxoethyl-2-hydroxy) amino]-D-*glycero*-D-*ido*-heptitol (**16**)

A stirred solution of methanol (MeOH) : NEt_3 : H_2O (5 : 1 : 1, 14 mL) was added acetyl derivative **15** (0.18 g, 0.28 mmol). After the mixture was stirred for 3 hr at room temperature, solvent was evaporated. The product was purified by column chromatography on silica gel (AcOEt) to afford 0.16 g (96.7%) of **16**, which was recrystallized from hexane–AcOEt: $[\alpha]_D$ + 56.5° (*c* 2.3, $CHCl_3$); mp 144–145°C; ^{1}H NMR ($CDCl_3$) δ 3.47–3.81 (m, 8H, H-1, H-3, H-4, H-5, H-6, and H-7), 3.96 (s, 2H, $-CH_2OH$), 4.10 (m, 1H, H-2), 4.44–4.90 (4 AB system, 8H, $-CH_2$-Ph), 6.62 (t, 1H, $J_{NH,\,1}$ 5.2 Hz, NH), 7.12–7.35 (m, 20H, aromatic H); MS: 612 (MH^+).

2,6-Anhydro-1-deoxy-1-[(1-oxoethyl-2-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**5**)

According to method F, compound **5** was prepared from **16** (80.0 mg, 0.13 mmol), the product was purified by column chromatography on silica gel (3 : 1 CH_2Cl_2–MeOH) to afford 32.4 mg (98.6%) of **5**: $[\alpha]_D$ + 20.0° (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD) δ 3.28 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.2 Hz, H-5), 3.54 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-4), 3.56–3.68 (m, 4H, H-1, H-6, and H-7a), 3.66 (dd, 1H, $J_{2,3}$ 5.6 Hz, H-3), 3.78 (dd, 1H, $J_{6,7b}$ 2.4 Hz, $J_{7a,7b}$ 11.6 Hz, H-7b), 3.99 (s, 2H, H-2′), 4.03 (ddd, 1H, $J_{2,3}$ 4.0 Hz, H-2); ^{13}C NMR (CD_3OD) δ 36.02 (C-1), 62.64 (C-2′), 62.87 (C-7), 71.87 (C-5), 72.38 (C-3), 75.16 (C-6), 75.31 (C-4), 75.82 (C-2), 175.30 (C-1′); HRMS: Anal. calcd for $C_9H_{18}NO_7 + H^+$; 252.1083. Found; 252.1095.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxopropyl-3-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**17**)

According to methods A and C, compound **17** was prepared from **13** (0.61 g, 1.11 mmol). The product was purified by column chromatography on silica gel (AcOEt) to afford 0.23 g (37.3%) of **17**, which was recrystallized from hexane–AcOEt: $[\alpha]_D$ + 55.0° (*c* 1.2, $CHCl_3$); mp 113–115°C; ^{1}H NMR ($CDCl_3$) δ 2.26 (td, 2H, $J_{2',3'}$ 5.8 Hz, $J_{3',OH}$ 1.6 Hz, H-3′), 3.42–3.81 (m, 10H, H-1, H-3, H-4, H-5, H-6, H-7, and H-2′), 4.08 (m, 1H, H-2), 4.45–4.91 (4 AB system, 8H, $-CH_2$-Ph), 6.09 (bs, 1H, NH), 7.14–7.35 (m, 20H, aromatic H); MS: 626 (MH^+).

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2,6-Anhydro-1-deoxy-1-[(1-oxopropyl-3-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**6**)

According to method F, compound **6** was prepared from **17** (60.1 mg, 96 μmol), the product was purified by column chromatography on silica gel (10 : 4 : 2 : 1 CH_2Cl_2–MeOH–EtOH–H_2O) to afford 20.1 mg (78.9%) of **6**: $[\alpha]_D + 66.7^\circ$ (*c* 1.0, MeOH); ^1H NMR (CD_3OD) δ 2.43 (t, 2H, $J_{2',3'}$ 6.4 Hz, H-3′), 3.23 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.0 Hz, H-5), 3.46–3.56 (m, 2H, H-1a and H-6), 3.52 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-4), 3.59–3.66 (m, 3H, H-1b, H-3, and H-7a), 3.79–3.82 (dd, 1H, H-7b), 3.80 (t, 2H, H-2′), 4.00 (ddd, 1H, $J_{1,2}$ 4.2 Hz, $J_{1,2}$ 10.2 Hz, $J_{2,3}$6.0 Hz, H-2); ^{13}C NMR (CD_3OD) δ 36.25 (C-1), 40.10 (C-3′), 59.43 (C-2′), 63.12 (C-7), 72.04 (C-5), 72.35 (C-3), 75.11 (C-6), 75.26 (C-4), 75.90 (C-2), 174.37 (C-1′); HRMS: Anal. calcd for $C_{10}H_{20}NO_7 + H^+$; 266.1240. Found; 266.1236.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxobutyl-4-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**18**)

According to methods A and C, compound **18** was prepared from **13** (0.52 g, 0.95 mmol). The product was purified by column chromatography on silica gel (AcOEt) to afford 0.23 g (41.3%) of **18**, which was recrystallized from hexane–AcOEt: $[\alpha]_D + 62.0^\circ$ (*c* 1.0, $CHCl_3$); mp 138–139°C; ^1H NMR ($CDCl_3$) δ 1.78 (tt, 2H, $J_{2',3'}$ 12.4 Hz, $J_{3',4'}$ 6.2 Hz, H-3′), 2.23 (td, 2H, $J_{4',\,OH}$ 1.4 Hz, H-4′), 3.40–3.80 (m, 10H, H-1, H-3, H-4, H-5, H-6, H-7, and H-2′), 4.09 (m, 1H, H-2), 4.45–4.91 (4 AB system, 8H, $-CH_2$-Ph), 6.21 (m, 1H, NH), 7.13–7.36 (m, 20H, aromatic H); MS: 640 (MH^+).

2,6-Anhydro-1-deoxy-1-[(1-oxobutyl-4-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**7**)

According to method F, compound **7** was prepared from **18** (168 mg, 0.26 mmol), the product was purified by column chromatography on silica gel (10 : 4 : 2 : 1 CH_2Cl_2–MeOH–EtOH–H_2O) to afford 72.1 mg (98.5%) of **7**: $[\alpha]_D + 70.0^\circ$ (*c* 0.8, MeOH); ^1H NMR (CD_3OD) δ 1.81 (tt, 2H, $J_{2',3'} = J_{3',4'}$ 6.8 Hz, H-3′), 2.30 (t, 2H, H-3′), 3.24 (dd, 1H, $J_{4,5} = J_{5,6}$ 8.8 Hz, H-5), 3.43–3.53 (m, 3H, H-1a, H-4, and H-6), 3.57 (t, 2H, H-2′), 3.59–3.66 (m, 3H, H-1b, H-3, and H-7a), 3.80 (dd, 1H, $J_{6,7b}$ 2.4 Hz, $J_{7a,7b}$ 11.6 Hz, H-7b), 3.98 (ddd, 1H, $J_{1,2}$ 4.0 Hz, $J_{1,2}$ 10.8 Hz, $J_{2,3}$ 5.6 Hz, H-2); ^{13}C NMR (CD_3OD) δ 29.80 (C-3′), 33.63 (C-4′), 36.22 (C-1), 62.26 (C-2′), 63.10 (C-7), 72.04 (C-5), 72.37 (C-3), 75.15 (C-6), 75.21 (C-4), 76.01 (C-2), 176.13 (C-1′); HRMS: Anal. calcd for $C_{11}H_{22}NO_7 + H^+$; 280.1396. Found; 280.1393.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-methoxycarbonyl) propylcarbonylamino]-D-*glycero*-D-*ido*-heptitol (**19**)

According to methods A and B, compound **19** was prepared from **13** (0.35 g, 0.63 mmol). The product was purified by column chromatography on silica gel (1 : 1 hexane–AcOEt) to afford 0.27 g (63.2%) of **19**, which was recrystallized from hexane–AcOEt: $[\alpha]_D + 38.7^\circ$ (*c* 1.5, $CHCl_3$); mp 113–115°C; ^1H NMR ($CDCl_3$) δ 2.11 (tt, 2H, $J_{2',3'} = J_{3',4'}$ 7.4 Hz, H-3′), 2.31 (t, 2H, H-2′), 3.44–3.78 (m, 10H, H-1, H-3, H-4, H-5, H-6,

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H-7, and H-4′), 3.65 (s, 3H, –OMe) 4.06 (m, 1H, H-2), 4.46–4.91 (4 AB system, 8H, $-CH_2$-Ph), 5.79 (m, 1H, NH), 7.13–7.36 (m, 20H, aromatic H); MS: 682 (MH^+).

2,6-Anhydro-3, 4, 5, 7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxopentyl-5-hydroxy) amino]-D-*glycero*-D-*ido*-heptitol (**20**)

According to method D, compound **20** was prepared from **19** (0.55 g, 0.81 mmol), the product was purified by column chromatography on silica gel (1 : 6 hexane–AcOEt) to afford 0.38 g (72.5%) of **20**, which was recrystallized from hexane–AcOEt: $[\alpha]_D + 45.3^\circ$ (*c* 1.5, $CHCl_3$); mp 130–131°C; ^{1}H NMR ($CDCl_3$) δ 1.53 (tt, 2H, $J_{3',4'} = J_{4',5'}$ 8.0 Hz, H-4′), 1.67 (tt, 2H, $J_{2',3'}$ 8.0 Hz, H-3′), 2.14 (t, 2H, H-5′), 3.41–3.80 (m, 10H, H-1, H-3, H-4, H-5, H-6, H-7, and H-2′), 4.09 (m, 1H, H-2), 4.45–4.91 (4 AB system, 8H, $-CH_2$-Ph), 6.04 (m, 1H, NH), 7.13–7.35 (m, 20H, aromatic H); MS: 654 (MH^+).

2,6-Anhydro-1-deoxy-1-[(1-oxopentyl-5-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**8**)

According to method F, compound **8** was prepared from **19** (269.8 mg, 0.41 mmol), the product was purified by column chromatography on silica gel (10 : 4 : 2 : 1 CH_2Cl_2–MeOH–EtOH–H_2O) to afford 92.6 mg (76.5%) of **8**: $[\alpha]_D + 36.4^\circ$ (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD) δ 1.55 (tt, 2H, H-3′), 1.67 (tt, 2H, H-4′), 2.24 (t, 2H, $J_{4',5'}$ 7.4 Hz, H-5′), 3.24 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.2 Hz, H-5), 3.43–3.53 (m, 2H, H-1a and H-6), 3.56 (dd, 1H, $J_{3,4}$ 9.0 Hz, H-4), 3.56 (t, 2H, $J_{2',3'}$ 6.4 Hz, H-2′), 3.59–3.67 (m, 3H, H-1b, H-3, and H-7a), 3.80 (dd, 1H, $J_{6,7b}$ 2.2 Hz, $J_{7a,7b}$ 11.8 Hz, H-7b), 3.98 (ddd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,2}$ 10.8 Hz, $J_{2,3}$ 5.8 Hz, H-2); ^{13}C NMR (CD_3OD) δ 23.41 (C-4′), 33.06 (C-3′), 36.17 (C-1), 36.76 (C-5′), 62.53 (C-2′), 63.09 (C-7), 72.02 (C-5), 72.37 (C-3), 75.15 (C-6), 75.20 (C-4), 76.07 (C-2), 176.32 (C-1′); HRMS: Anal. calcd for $C_{12}H_{24}NO_7 + H^+$; 294.1553. Found; 294.1556.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-methoxycarbonyl) butylcarbonylamino]-D-*glycero*-D-*ido*-heptitol (**21**)

According to methods A and B, compound **21** was prepared from **13** (0.45 g, 0.81 mmol). The product was purified by column chromatography on silica gel (1 : 1 hexane–AcOEt) to afford 0.12 g (21.3%) of **21**, which was recrystallized from hexane–AcOEt: $[\alpha]_D + 39.0^\circ$ (*c* 2.0, $CHCl_3$); mp 104–105°C; ^{1}H NMR ($CDCl_3$) δ 1.60 (tt, 2H, $J_{2',3'} = J_{3',4'}$ 7.2 Hz, H-3′), 2.06 (tt, 2H, $J_{4',5'}$ 6.8 Hz, H-4′), 2.28 (t, 2H, H-2′), 3.43–3.79 (m, 10H, H-1, H-3, H-4, H-5, H-6, H-7, and H-5′), 3.65 (s, 3H, –OMe), 4.07 (m, 1H, H-2), 4.46–4.91 (4 AB system, 8H, $-CH_2$-Ph), 5.78 (m, 1H, NH), 7.13–7.32 (m, 20H, aromatic H); MS: 696 (MH^+).

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-1-[(1-oxohexyl-6-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**22**)

According to method D, compound **22** was prepared from **21** (0.214 g, 0.31 mmol), the product was recrystallized from hexane–AcOE to afford 0.184 g (89.6%) of **22**: $[\alpha]_D + 50.1^\circ$ (*c* 1.7, $CHCl_3$); mp 152–153°C; ^{1}H NMR ($CDCl_3$) δ 1.33 (tt, 2H, $J_{3',4'} = J_{4',5'}$ 7.6 Hz, H-4′), 1.52 (tt, 2H, $J_{5',6'}$ 7.4 Hz, H-5′), 1.60 (tt, 2H, $J_{2',3'}$ 7.6 Hz, H-3′),

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2.09 (t, 2H, H-6′), 3.37–3.78 (m, 10H, H-1, H-3, H-4, H-5, H-6, H-7, and H-2′), 4.08 (m, 1H, H-2), 4.46–4.94 (4 AB system, 8H, $-CH_2$-Ph), 5.92 (m, 1H, NH), 7.14–7.34 (m, 20H, aromatic H); MS: 668 (MH^+).

2,6-Anhydro-1-deoxy-1-[(1-oxohexyl-6-hydroxy)amino]-D-*glycero*-D-*ido*-heptitol (**9**)

According to method F, compound **9** was prepared from **22** (132 mg, 0.20 mmol), the product was purified by column chromatography on silica gel (10 : 4 : 2 : 1 CH_2Cl_2–MeOH–EtOH–H_2O) to afford 60.4 mg (99.3%) of **9**: $[\alpha]_D + 88.6^\circ$ (*c* 0.7, MeOH); ^{1}H NMR (CD_3OD) δ 1.39 (tt, 2H, H-4′), 1.54 (tt, 2H, H-3′), 1.64 (tt, 2H, H-5′), 2.24 (t, 2H, $J_{5',6'}$ 7.6 Hz, H-6′), 3.25 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.2 Hz, H-5), 3.43–3.53 (m, 3H, H-1a, H-4, and H-6), 3.55 (t, 2H, $J_{2',3'}$ 6.4 Hz, H-2′), 3.56–3.67 (m, 3H, H-1b, H-3, and H-7a), 3.78 (dd, 1H, $J_{6,7b}$ 2.0 Hz, $J_{7a,7b}$ 12.0 Hz, H-7b), 3.98 (ddd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,2}$ 11.0 Hz, $J_{2,3}$ 5.8 Hz, H-2); ^{13}C NMR (CD_3OD) δ 26.54 (C-4′), 26.84 (C-5′), 33.32 (C-3′), 36.17 (C-7), 37.04 (C-6′). 62.77 (C-2′), 63.07 (C-1), 72.02 (C-5), 72.34 (C-3), 75.13 (C-6), 75.18 (C-4), 76.03 (C-2), 176.41 (C-1′); HRMS: Anal. calcd for $C_{13}H_{26}NO_7 + H^+$; 308.1709. Found; 308.1702.

1-Acetamide-2,6-anhydro-D-*glycero*-D-*ido*-heptitol (**10**)

According to method F, compound **10** was prepared from **23** (0.16 mg, 0.27 mmol),[9] the product was purified by column chromatography on silica gel (10 : 4 : 2 : 1 CH_2Cl_2–MeOH-EtOH–H_2O) to afford 63.2 mg (98.9%) of **6**: $[\alpha]_D + 26.0^\circ$ (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 1.96 (s, 3H, $COCH_3$), 3.24 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.0 Hz, H-5), 3.45 (dd, 1H, $J_{6,7a}$ 11.2 Hz, $J_{7a,7b}$ 14.4, H-7a), 3.51 (dd, 1H, $J_{3,4}$ 9.0 Hz, H-4), 3.54 (m, 1H, H-6), 3.59–3.64 (m, 3H, H-1 and H-7a), 3.65 (dd, 1H, $J_{2,3}$ 6.2 Hz, H-3), 3.80 (dd, 1H, $J_{7a,7b}$ 2.4 Hz, $J_{6,7b}$ 11.6 Hz, H-7b), 3.98 (ddd, 1H, $J_{1a,2}$ 3.6 Hz, H-2); ^{13}C NMR (CD_3OD) δ 22.56 (CH_3), 36.26 (C-1), 63.09 (C-7), 72.02 (C-5), 72.30 (C-3), 75.08 (C-4), 75.20 (C-6), 76.00 (C-2), 173.75 (C═O); HRMS: Anal. calcd for $C_9H_{18}NO_6 + H^+$; 236.1134. Found; 236.1135.

2,6-Anhydro-1-benzamide-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*ido*-heptitol (**24**)

According to methods A and E, compound **24** was prepared from **13** (0.54 g, 0.98 mmol), the product was purified by column chromatography on silica gel (AcOEt) to afford 0.38 mg (70.4%) of **24**, which was recrystallized from hexane–AcOEt: $[\alpha]_D + 68.3^\circ$ (*c* 1.2, $CHCl_3$); mp 141–143°C; ^{1}H NMR ($CDCl_3$) δ 3.51 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.0 Hz, H-5), 3.59 (dd, 1H, $J_{6,7a}$ 5.4 Hz, $J_{7a,7b}$ 10.2 Hz, H-7a), 3.67 (dd, 1H, $J_{6,7b}$ 2.0 Hz, H-7b), 3.68–3.87 (m, 4H, H-1a, H-3, H-4, and H-6), 3.97 (ddd, 1H, $J_{1a,1b}$ 14.8 Hz, $J_{1b,2}$ 6.4 Hz, $J_{1b,NH}$ 5.0 Hz, H-1b), 4.21 (ddd, 1H, H-2), 4.47–4.92 (4 AB system, 8H, $-CH_2$-Ph), 6.55 (bs, 1H, NH), 7.14–7.71 (m, 24H, aromatic H); MS: 658 (MH^+).

2,6-Anhydro-1-benzamide-D-*glycero*-D-*ido*-heptitol (**11**)

According to method F, compound **11** was prepared from **24** (0.20 g, 0.30 mmol), the product was purified by column chromatography on silica gel (5 : 1 CH_2Cl_2–MeOH) to

afford 77.6 mg (87.9%) of **11**: $[\alpha]_D + 107.5^\circ$ (*c* 0.8, MeOH); ^{1}H NMR (CD_3OD) δ 3.23 (dd, 1H, $J_{4,5} = J_{5,6}$ 9.4 Hz, H-5), 3.53 (dd, 1H, $J_{3,4}$ 9.0 Hz, H-4), 3.58–3.65 (m, 3H, H-3, H-6, and H-7a), 3.66 (dd, 1H, $J_{1a,1b} = J_{1a,2}$ 10.8 Hz, H-1a), 3.74–3.79 (m, 2H, H-1b and H-7b), 4.10 (ddd, 1H, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 6.5 Hz, H-2), 7.38–7.79 (m, 4H, aromatic H); ^{13}C NMR (CD_3OD) δ 36.88 (C-1), 63.04 (C-7), 72.06 (C-5), 72.42 (C-3), 75.25 (C-4 and C-6), 76.06 (C-2), 128.34, 129.52, 132.62, and 135.78 (aromatic C), 170.64 (C═O); HRMS: Anal. calcd for $C_{14}H_{20}NO_6 + H^+$; 298.1291. Found; 298.1287.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-trimethylacetamide-D-*glycero*-D-*ido*-heptitol (**25**)

According to methods A and E, compound **25** was prepared from **13** (0.61 g, 1.10 mmol), the product was purified by column chromatography on silica gel (AcOEt) to afford 0.65 g (72.3%) of **25**: $[\alpha]_D + 35.6^\circ$ (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.12 (s, 9H, $C(CH_3)_3$), 3.42–3.83 (m, 8H, H-1, H-3, H-4, H-5, H-6 and H-7), 4.11 (ddd, 1H, $J_{1a,2} = J_{2,3}$ 4.8 Hz, $J_{1b,2}$ 9.6 Hz, H-2), 4.46–4.91 (4 AB system, 8H, $-CH_2-$Ph), 5.98 (bs, 1H, NH), 7.14–7.35 (m, 20H, aromatic H); MS: 638 (MH^+).

2,6-Anhydro-1-trimethylacetamide-D-*glycero*-D-*ido*-heptitol (**12**)

According to method F, compound **12** was prepared from **25** (0.17 g, 0.26 mmol), the product was purified by column chromatography on silica gel (4 : 1 CH_2Cl_2–MeOH) to afford 64.8 mg (89.1%) of **12**: $[\alpha]_D + 105.0^\circ$ (*c* 0.8, MeOH); ^{1}H NMR (CD_3OD) δ 1.18 (s, 9H, 3 × CH_3), 3.25 (dd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6}$ 8.8 Hz, H-5), 3.50–3.56 (m, 4H, H-4, H-6, and H-1), 3.63 (dd, 1H, $J_{2,3}$ 6.0 Hz, $J_{3,4}$ 9.6 Hz, H-3), 3.65 (dd, 1H, $J_{7a,7b}$ 2.0 Hz, $J_{6,7a}$ 6.0 Hz, H-7a), 3.77 (dd, $J_{6,7b}$ 11.8 Hz, H-7b), 4.00 (ddd, 1H, H-2); ^{13}C NMR (CD_3OD) δ 27.82 (3 × CH_3), 36.69 (C-1), 39.74 ($C(CH_3)_3$), 62.95 (C-7), 72.00 (C-5), 72.55 (C-3), 75.28 (C-4 and C-6), 75.71 (C-2), 181.59 (C═O); HRMS: Anal. calcd for $C_{12}H_{24}NO_6 + H^+$; 278.1604. Found; 278.1601.

Enzymatic Assays

p-Nitrophenyl α-D-glucopyranoside was purchased from Sigma–Aldrich Inc. [^{3}H]Glucose labeled VSV G was prepared as previously reported methods.[15,18,19] Rat liver microsomes were prepared following the previously reported methods, and membranes were collected by centrifugation at 12,000*g* for 1 hr and resuspended in 100 mM potassium phosphate buffer (pH 6.8) containing 10 mM glycerol, 2 mM $MgCl_2$, 0.5 μM DTT.[15] Activities of processing α-glucosidase I and α-glucosidase II were assayed using rat liver microsomes as an enzyme source. In assays of the inhibitory effects of PNP Glc and probes **1**–**12** against processing α-glucosidase I, we used [^{3}H]glucose labeled VSV G as substrate. The hydrolytic activities of the enzyme was assayed by measuring the release of [^{3}H]glucose under 100 mM potassium phosphate buffer (pH 6.8) containing 20 mM EDTA, 0.02% NaN_3, 0.05% Triton X-100, and protease inhibitor cocktails at 37°C. The processing α-glucosidase II assays were performed by using PNP Glc as substrate and were assayed the previously reported methods,[11–14] the assay condition was 100 mM potassium phosphate buffer containing 0.05% Triton X-100 (pH 6.8) at 37°C. One unit of enzyme activity was defined as

the amount of enzyme required to liberate 1 μmol of *p*-nitrophenol/min at each assay condition. Kinetic study on the hydrolysis of PNP Glc and **1** by α-glucosidase II was performed at each concentration, and values of K_m (mM) and V_{max} (μmol/min/unit) were calculated from reciprocal plots of their reaction curves.

Inhibition Assays at Cellular Level

Confluent monolayer of BHK cells are infected with the VSV, and nectrisine (a final concentration 10 μg/mL, Nectrisine was kindly supplied from Fujisawa Pharmaceutical Co., Tokyo, Japan), probes **5**–**10** and **12** (final concentrations 100 μg/mL), and probe **11** (a final concentration 500 μg/mL) are added to infected cultures as described.[18–20] The obtained VSV G proteins were digested with endo H by reported method.[18] The treated VSV G proteins were subjected to SDS-polyacrylamide gel electrophoresis using 10% acryl amide gel, followed by electrophoretic transfer onto nitrocellulose membrane. The protein replica was incubated with rabbit polyclonal antibodies raised against VSV G.[18] Antigen–antibody complexes were detected with a goat anti-rabbit IgG antibody–alkaline phosphatase conjugate by staining with 5-bromo-4-chloro-3-indolyl phosphate.

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